Enzymatic Assay of ARYL ACYLAMIDASE
(EC 3.5.1.13)

PRINCIPLE:

N-Acetyl-p-Aminophenol + H_2O $\xrightarrow{\text{Aryl Acylamidase}}$ p-Aminophenol + Acetate

CONDITIONS: T = 37°C, pH = 9.0, A_{615nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 9.0 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 9.0 at 37°C with 1 M HCl.)

B. 100 mM N-Acetyl-p-Aminophenol Solution (NAAP)
(Prepare 50 ml in Reagent A using 4-Acetamidophenol, Sigma Prod. No. A-5000.)

C. 5.0 mM p-Aminophenol Standard Solution (Std)
(Prepare by dissolving 54.56 mg of p-Aminophenol, Sigma Prod. No. A-4076, in 80 ml of deionized water. Adjust the pH to 11.0 at 25°C with 0.1 M NaOH. The solution should be clear and purple at this point. Adjust the pH back to 9.0 using 0.1 M HCl. The color of the solution should turn brown. Quantitatively transfer to a 100 ml volumetric flask and dilute to 100 ml with deionized water. Protect the solution from light and use immediately after preparation.)

D. 270 mM Ammonium Hydroxide Solution (NH_4OH)
(Prepare 100 ml in deionized water using Ammonium Hydroxide, Sigma Prod. No. A-6899.)

E. 1.28 mM Cupric Sulfate Solution ($CuSO_4$)
(Prepare 100 ml in Reagent D using Cupric Sulfate, Pentahydrate, Sigma Prod. No. C-7631.)

F. 0.41% (v/v) o-Cresol Solution
(Prepare 48.2 ml by adding 0.2 ml of o-Cresol, Sigma Prod. No. C-7400 to 48 ml of deionized water. **PREPARE FRESH**.)

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REAGENTS: (continued)

G. Color Reagent Solution (CRS)
(Prepare 54.2 ml by adding 48.2 ml of Reagent F to 6 ml of Reagent E. Mix well. Prepare fresh and use within 30 minutes.)

H. Aryl Acylamidase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.13 - 0.25 unit/ml of Aryl Acylamidase in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	~~Test~~	~~Blank~~
Reagent A (Buffer)	------	0.10
Reagent B (NAAP)	2.90	2.90

Mix by swirling and equilibrate to 37°C. Then add:

Reagent H (Enzyme Solution)	0.10	------

Immediately mix by inversion and incubate at 37°C for exactly 10 minutes.

Pipette (in milliliters) the following reagents into suitable containers:

Reagent G (CRS)	2.50	2.50
Test Mixture	1.00	------
Blank Mixture	------	1.00

Mix by swirling and incubate at room temperature for exactly 5 minutes. Transfer the solutions to suitable cuvettes and record the A_{615nm} for the Test and Blank using a suitable spectrophotometer.

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

	~~Std 1~~	~~Std 2~~	~~Std 3~~	~~Std 4~~	~~Std 5~~	Std ~~Blank~~
Reagent C (Std)	0.02	0.04	0.06	0.08	0.10	---
Deionized Water	0.08	0.06	0.04	0.02	---	0.10
Reagent B (NAAP)	2.90	2.90	2.90	2.90	2.90	2.90

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PROCEDURE: (continued)

Mix by swirling and incubate at 37°C for exactly 10 minutes.

Pipette (in milliliters) the following reagents into suitable containers:

	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Reagent G (CRS)	2.50	2.50	2.50	2.50	2.50	2.50
Std 1	1.00	---	---	---	---	---
Std 2	---	1.00	---	---	---	---
Std 3	---	---	1.00	---	---	---
Std 4	---	---	---	1.00	---	---
Std 5	---	---	---	---	1.00	---
Blank	---	---	---	---	---	1.00

Mix by swirling and incubate at room temperature for exactly 5 minutes. Transfer the solutions to suitable cuvettes and record the A_{615nm} for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

r A_{615nm} Standard = A_{615nm} Standard - A_{615nm} Standard Blank

Plot the rA_{615nm} Standard vs µmoles of p-Aminophenol.

Sample Determination:

r A_{615nm} Sample = A_{615nm} Test - A_{615nm} Test Blank.

Determine the µmoles of p-Aminophenol liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\text{µmoles of p-Aminophenol released})(df)}{(10)(0.1)}$$

df = Dilution factor
10 = Time (in minutes) of assay as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used in the assay

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

CALCULATIONS: (continued)

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will convert 1.0 µmole of N-acetyl-p-aminophenol (acetaminophen) to p-aminophenol per minute at pH 9.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 100 mM Tris, 97 mM N-acetyl-p-aminophenol, and 0.0125 - 0.025 unit aryl acylamidase.

REFERENCE:

Price, C.P., Hammond, P.M., and Scawen, M.D. (1983) *Clinical Chemistry* **29**, 358-361

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.